ELSEVIER

Virus Research 48 (1997) 143–148

Virus Research

Evidence that fatal human infections with La Crosse virus may be associated with a narrow range of genotypes

Cinnia Huang [a,*], Wayne H. Thompson [b], Nick Karabatsos [c], Leo Grady [a], Wayne P. Campbell [a]

[a] *Wadsworth Center, New York State Department of Health, P.O. Box 509, Albony, NY 12201-0509, USA*
[b] *2880 North Road, Blue mounds, WI 53517-9695, USA*
[c] *Center for Disease Control and Prevention, P.O. Box 2087/CSU Foothills Campus Fort Collins, CO 80522, USA*

Received 23 September 1996; received in revised form 12 December 1996; accepted 14 December 1996

Abstract

La Crosse (LAC) virus belongs to the California (CAL) serogroup of the genus *Bunyavirus*, family *Bunyaviridae*. It is considered one of the most important mosquito-borne pathogens in North America, especially in the upper Mid-West, where it is associated with encephalitis during the time of year when mosquitoes are active. Infections occur most frequently in children and young adults and, while most cases are resolved after a period of intense illness, a small fraction ($< 1\%$) are fatal. At present there have only been three isolates of LAC virus from humans, all made from brain tissue postmortem. The cases yielding viruses are separated chronologically by 33 years and geographically from Minnesota/Wisconsin (1960, 1978) to Missouri (1993). The M RNA sequence of the first two isolates was previously reported. The present study extends the observations to the isolate from the 1993 case and includes several mosquito isolates as well. A comparison of the M RNAs of these viruses shows that for the human isolates both nucleotide sequence and the deduced amino-acid sequence of the encoded proteins are highly conserved, showing a maximum variation of only 0.91% and 0.69%, respectively. This high degree of conservation over time and space leads to the hypothesis that human infections with this particular genotype of LAC virus are those most likely to have a fatal outcome. It is also shown that a virus with this genotype could be found circulating in mosquitoes in an area more or less intermediate between the locations of the first and second fatal cases. © 1997 Elsevier Science B.V.

Keywords: La Crosse virus; Mosquito isolates; Genotype; RNA

* Corresponding author. Fax: +1 518 8696487; e-mail: cinnia.huang@wadsworth.org

The history, epidemiology, and molecular biology of the CAL serogroup viruses have been reviewed in depth (Calisher, 1983; Elliott, 1996). Briefly, the virion contains four structural

PII S0168-1702(97)01437-8

Table 1
Summary of LAC virus isolates used in our studies

Virus isolate	Source	Location and date of origin	Passage history[a]	Accession no.	Reference
LAC30928-31	Homo sapiens	Dresbach, MN, 1960[b]	SMB3; BHK3	U18979	Huang et al. (1995)
LAC22988-89	Homo sapiens	De Soto, WI, 1978	SMB2; BHK2	U18980	Huang et al. (1995)
LACR56869	Homo sapiens	Stone County, MO, 1993	Vero1	U70205	This paper
LAC76-40	Ae. triseriatus	Richland County, WI, 1976	SMB2; BHK1	U70206	This paper
LAC79-283	Ae. triseriatus	Crawford County, WI, 1979	SMB2; BHK1	U70207	This paper
LAC81-4	Ae. triseriatus	Washington County, WI, 1981	SMB2; BHK1	U70208	This paper
LAC74-32813	Ae. triseriatus	Albany County, NY, 1974	SMB4; BHK4	D10370	Grady et al. (1987)

[a] SMB, suckling mouse brain; BHK, baby hamster kidney cells; Vero, Vero cells
[b] Site of human infection. The transport for medical care accounts for the isolation of the virus in La Crosse, Wisconsin.

proteins: two external glycoproteins (G1 and G2), a nucleocapsid (N) protein, and a large (L) protein (Obijeski et al., 1976a; Gentsch and Bishop, 1978, 1979; Cash et al., 1979; Fuller and Bishop, 1982; Fuller et al., 1983). The viral genome consists of three single-stranded, negative-sense RNA segments: L (large); M (middle), and S (small) (Obijeski et al., 1976b). The L RNA encodes the viral RNA-directed RNA polymerase, the S RNA codes for the nucleocapsid (N) protein and a non-structural protein (NS_S), and the M RNA encodes the two envelope glycoproteins (G2 and G1) and a non-structural protein (NS_M) with unknown function (Endres et al., 1989; Gentsch and Bishop, 1978; Cash et al., 1979; Fuller and Bishop, 1982; Fuller et al., 1983; Elliott, 1985; Gentsch and Bishop, 1979; Fazakerly et al., 1988). Although these viruses were first described in 1952, their importance in human CNS infections only began to be fully appreciated following the isolation of LAC virus in 1960 (Hammon and Reeves, 1952; Thompson et al., 1965).

The importance of the M RNA in pathogenicity of these viruses was established in studies employing reassortant viruses (Shope et al., 1981). It was later shown that neuropathogenicity was polygenic, with neuroinvasiveness being ascribed to the M RNA and neurovirulence being mapped to the L RNA (Gonzalez-Scarano et al., 1992).

The initial observation of genetic similarity between the first two human isolates was based on a comparison of their RNase T1 fingerprints (Klimas et al., 1981). This has since been confirmed by sequencing their M RNAs and extended to include the deduced amino-acid sequence of their G1 and G2 glycoproteins (Huang et al., 1995). However, while separated in time by 18 years, both of these viruses came from the same general area and there was no basis for evaluating the contribution that geographic proximity might make to their genetic similarity. This is an important consideration since the RNase T1 fingerprinting studies showed significant variation among independent mosquito isolates of LAC virus that seemed, at least partly, to be influenced by the location of origin (El Said et al., 1979; Klimas et al., 1981).

In 1993, Karabatsos recovered LAC virus from a fatal case of encephalitis that occurred that year in Missouri. This provided a unique opportunity to obtain sequence data on a third human isolate of the virus that was separated both spatially and in time from those studied previously (by approximately 400 miles and by 33 and 15 years). As part of the study, data were also obtained for several Wisconsin mosquito isolates of LAC virus. This report summarizes the results of that investigation.

The geographical location, year of isolation, source of origin, and passage history of the LAC isolates used are listed in Table 1. Except for the third human isolate, LACR56869, BHK-21 cells were used to prepare stock virus as described previously (Campbell and Huang, 1995). For LACR56869, viral sequences were amplified, cloned, and sequenced directly from the material received from Fort Collins, i.e. the virus was never grown in the laboratory where the sequence analysis was performed. Total RNA was extracted

from 20 μl of stock virus using Ultraspec RNA (Biotecx Laboratories) and random hexamers (BMB) were used to transcribe RNA to cDNA as previously described (Campbell and Huang, 1995). An aliquot of the cDNA reaction mixture (2–5 μl) was added to a 50 μl polymerase chain reaction (PCR) reaction mixture containing appropriate primer pairs. After PCR amplification, the sequences were either cloned into a plasmid vector and sequenced or alternately, the RT-PCR products were sequenced directly. All sequence data have been deposited in the GenBank sequence database (accession numbers: U70205–U70208).

Sequence analyses were performed using the program devised by the University of Wisconsin Genetic Computer Group (UWGCG) (Devereux et al., 1984). Consistent with earlier reports, the M RNA of all LAC isolates examined contained 4526 nucleotides, with a single, long open reading frame (ORF) in the viral complementary strand (Grady et al., 1987; Huang et al., 1995). The cysteine residues, except the one in the signal sequence of G2, and five potential *N*-glycosylation sites were conserved among all seven isolates of LAC virus (Fig. 1).

Table 2 summarizes the main features that emerge from a comparison of the LAC M RNAs

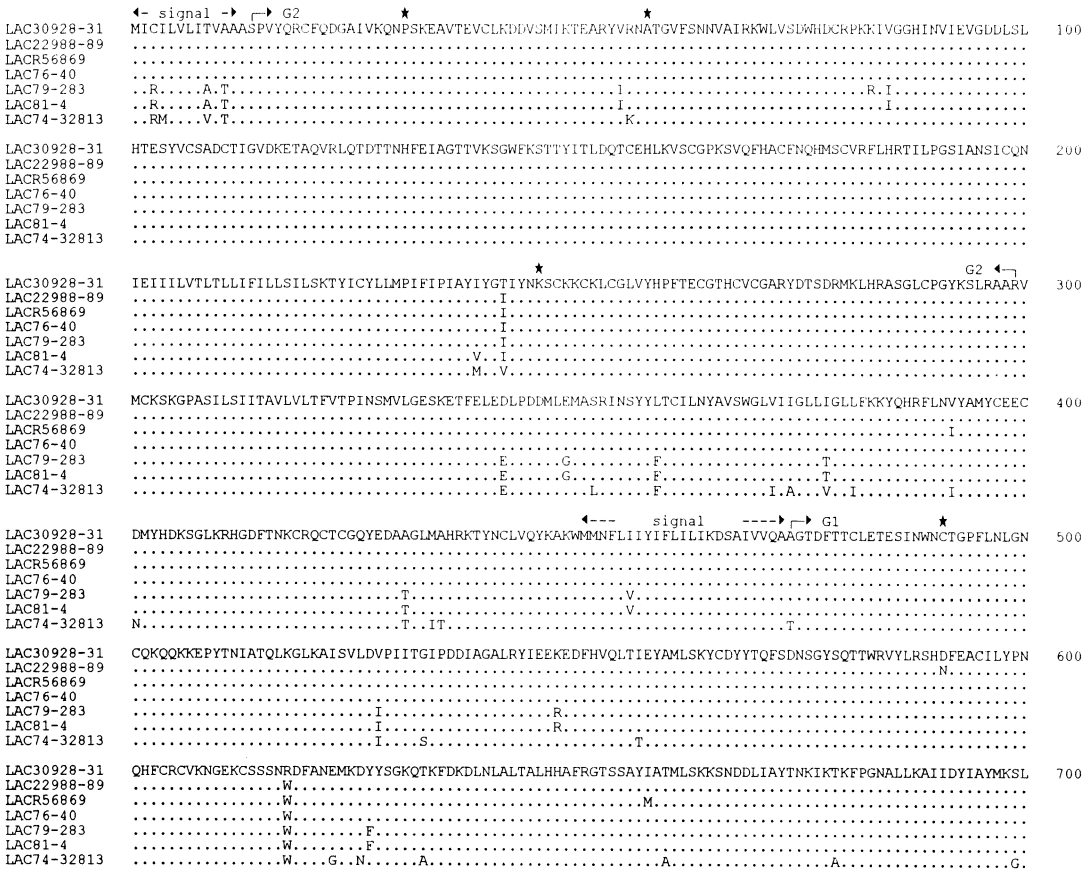

Fig. 1. Deduced amino-acid sequences of M RNA genome of 7 LAC virus isolates. Alignment of the sequences was generated by the PUBLISH program in the UWGCG package. Complete data are shown for LAC30928-31, the prototype of LAC virus. For the other isolates, dots indicate identity with LAC30928-31 and amino acids which differ are given. The signal sequences of the amino terminus for both G1 and G2, as well as the N-linked glycosylation sites (✱) are indicated.

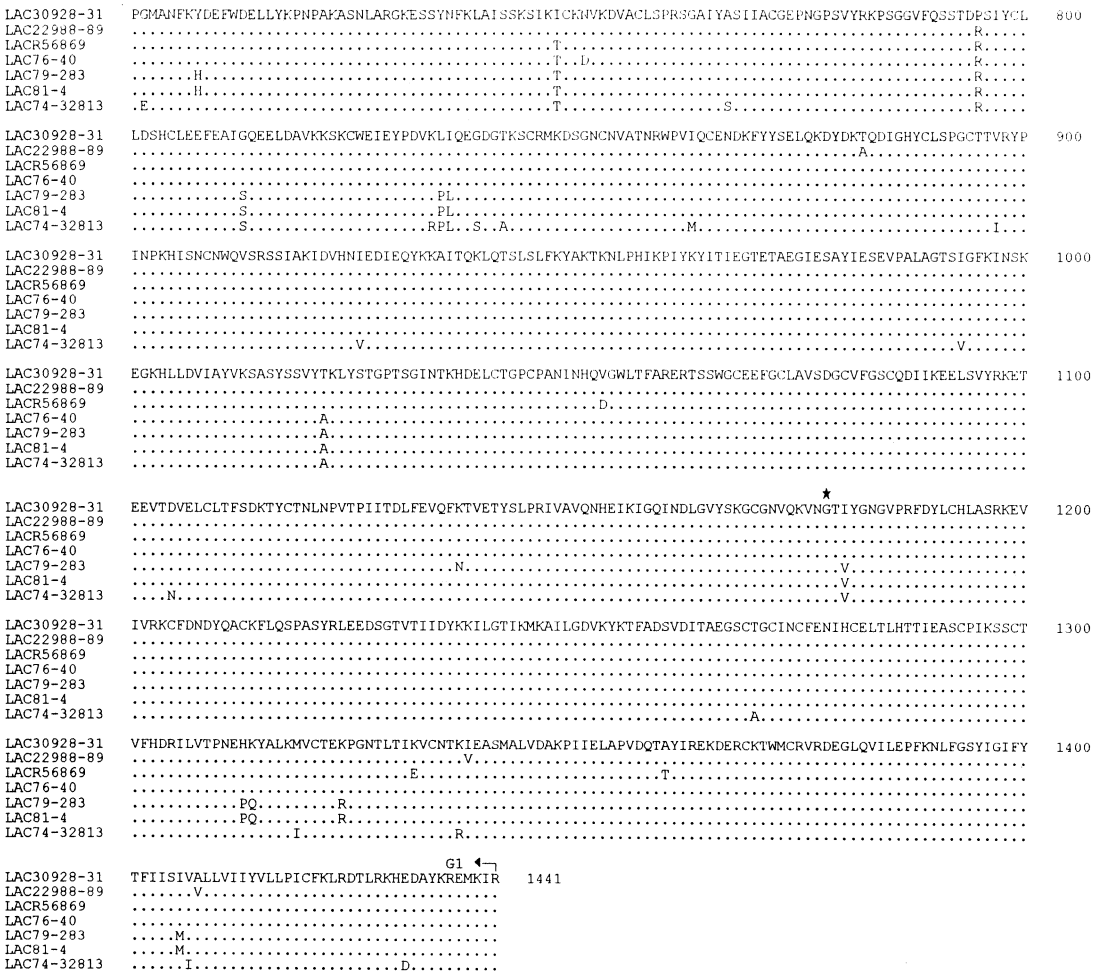

Fig. 1 (contd.)

sequenced in this study. With regard to the human isolates, all of the nucleotide differences occurred within the ORF. There were a total of 51 different sites affected, which corresponds to 98.9% nucleotide sequence conservation among the three human isolates. In terms of amino-acid sequence, there were no changes found in the first 13 residues which comprise the leader sequence. In the region coinciding with G2, a single change from T to I at position 242 was detected for both LAC22988-89 and LACR56869. There is a single change in the region corresponding to the non-structural protein (NS_M) for LACR56869, whereas the region associated with G1 had 11 sites affected. Two of these sites were identical in LAC22988-89 and LACR56869. Four other sites were unique to the former and five were unique to the latter. Thus amino-acid sequence conservation among the three human isolates was 100% in the leader sequence, 99.7% in the G2 protein, 99.4% in NS_M, and 98.9% in the G1 protein. On the basis of these data and in view of the geographical and chronological distances between these viruses, it seems reasonable to hypothesize that there are strong selection pressures operating with respect to the ability to produce fatal human infection.

Assuming that the small proportion of LAC infections with fatal outcomes are attributable to

Table 2
Number of nucleotide and amino acid sequence differences with respect to the prototype strain of LAC virus (LAC30928-31)

Virus isolate	Nucleotide		Amino Acid			
	M RNA (1–4526)	ORF (62–4387)	aa (1–1441)	Leader (aa 1–13)	G2 (14–299)	G1 (474–1441)
LAC22988-89	20	20	7	0	1	6
LACR56869	41	41	9	0	1	7
LAC76-40	34	33	6	0	1	5
LAC79-283	241	233	30	3	4	17
LAC81-4	244	236	29	3	4	16
LAC74-32813	395	387	52	4	3	33

infection with a particular variant, we can visualize at least two scenarios on how such infections might arise: (1) the genotype in question could be selected at some stage postinfection; or (2) the genotype is present as one of many that are naturally circulating and chance determines whether someone is unfortunate enough to be bitten by a mosquito carrying it.

The former hypothesis is not readily tested in a natural setting, but there are laboratory data which tend to argue against it. These consist of the demonstration that the viral genome shows great stability when passed from mosquito to mouse, as well as when passed transovarially from one mosquito generation to the next (Baldridge et al., 1989).

An attempt to explore the second hypothesis was initiated by focusing on some mosquito isolates of LAC virus from Wisconsin that were obtained in the same general time-frame as the 1978 fatal human case. As can be seen in Table 2, LAC76-40, one of the three isolates examined, had an M RNA sequence that closely correlated with the viruses recovered from humans. Although it was obtained 2 years before the 1978 human case, it came from a geographic area almost midway between the sites of the 1960 and 1978 cases. LAC76-40 contained only two amino-acid changes that were not reflected among the human isolates, both in the G1 glycoprotein. One of these was unique to LAC76-40 and the other was found in all of the mosquito isolates, including that from New York.

There were 13 nucleotide differences (99.7% identity) between two Wisconsin mosquito isolates LAC79-283 and LAC81-4, which results in the prediction of three amino-acid changes. Two of these predicted differences occur in G2 and one in G1 glycoprotein. Both sequence data of LAC79-283 and LAC81-4 showed homology of 94.7% to the prototype strain of LAC virus. These two isolates and the mosquito isolate from New York represent different genotypes of LAC virus (Table 2).

Klimas et al. (1981) observed that the 1965 Spring Valley isolate, which was obtained from a pool of *Ae. triseriatus* in the yard of 1960 fatal case, was almost identical to the prototype virus. However, the exact relationships and the number of mutations were not clearly demonstrated. At the very least, our finding in this study shows that the genotype associated with human fatalities also circulates in mosquitoes. Nevertheless, it does not preclude that selection could also occur after infection, nor does it provide any information on whether other factors exist that could ameliorate infection with this variant. These results do, however, suggest that augmenting surveillance for LAC virus with sequencing might be useful and that detection of this variant in a given locale might justify particularly aggressive mosquito-control measures.

Acknowledgements

We thank the Molecular Genetic core facility for kindly providing us with the oligonucleotides and automated sequencing.

References

Baldridge, G.D., Beaty, B.J. and Hewlett, M.J. (1989). Genomic stability of La Crosse virus during vertical and horizontal transmission. Arch. Virol. 108, 89–99.

Calisher, C.H. (1983) Taxonomy, Classification, and geographic distribution of California serogroup bunyaviruses: In: C. H. Calisher and W.H. Thompson (Eds.) California Serogroup Viruses, Liss, New York, pp. 1–16.

Campbell, W.P. and Huang, C. (1995) Detection of California serogroup viruses using universal primers and reverse transcription-polymerase chain reaction. J. Virol. Methods 53, 55–61.

Cash, P., Vezza, A.C., Gentsch, J.R. and Bishop, D.H.L. (1979) Genome complexities of the 3 mRNA species of snowshoe hare bunyavirus and in vitro translation of S mRNA to viral N polypeptide. J. Virol. 31, 685–694.

Devereux, J., Haeberli, P. and Smithies, O. (1984) A comprehensive set of sequence analysis program for the VAX. Nucleic Acids Res. 12, 387–395.

El Said, L.H., Vorndam, V., Gentsch, J.R., Clewley, J.P., Calisher, C.H., Klimas, R.A., Thompson, W.H., Grayson, M., Trent, D.W. and Bishop, D.H.L. (1979) A comparison of La Crosse virus isolates obtained from different ecological niches and an analysis of the structural components of California encephalitis serogroup viruses and other bunyaviruses. Am. J. Trop. Med. Hyg. 28, 364–386.

Elliott, R.M. (1985) Identification of non-structural proteins encoded by viruses of the Bunyamwera serogroup (family Bunyaviridae). Virology 143, 119–126.

Elliott, R.M. (ed.). The Bunyaviridae, Plenum Press, New York, 1996.

Endres, M.J., Jacoby, D.R., Jassen, R.S., Gonzalez-Scarano, F. and Nathanson, N. (1989) The large viral RNA segment of California serogroup Bunyaviruses encodes the large viral protein. J. Gen. Virol. 70, 223–228.

Fazakerly, J.K., Gonzalez-Scarano, F., Strickler, J., Dietzschold, B., Karush, F. and Nathanson, N.(1988) Organization of the middle RNA segment of snowshoe hare bunyavirus. Virology 167, 422–432.

Fuller, F. and Bishop, D.H.L. (1982) Identification of virus-coded nonstructural polypeptides in bunyavirus-infected cells. J. Virol. 41, 643–648.

Fuller, F., Brown, A.S. and Bishop, D.H.L. (1983) Bunyavirus nucleoprotein, N, and a non-structural protein, NS_s, are coded by overlapping reading frames in the S RNA. J. Gen. Virol. 64, 1705–1714.

Gentsch, J.R. and Bishop, D.H.L. (1978) Small viral RNA segment of bunyaviruses codes for viral nucleocapsid protein. J. Virol. 28, 417–419.

Gentsch, J.R. and Bishop, D.H.L. (1979) M viral RNA segment of bunyaviruses codes for 2 glycoproteins, G1 and G2. J. Virol. 30, 767–770.

Gonzalez-Scarano, F., Jacoby, D., Griot, C. and Nathanson, N. (1992) Genetics, infectivity and virulence of California serogroup viruses. Virus Res. 24, 123–135.

Grady, L.J., Sanders, M.L. and Campbell, W.P.(1987) The sequence of the M RNA of an isolate of La Crosse virus. J. Gen. Virol. 68, 3057–3071.

Hammon, W.McD. and Reeves,W.C. (1952) California encephalitis virus, a newly described agent. Calif. Med. 77, 303–309.

Huang, C., Thompson, W.H. and Campbell, W.P. (1995) Comparison of the M RNA genome segments of 2 human isolates of La Crosse virus. Virus Res. 36, 177–185.

Klimas, R.A., Thompson, W.H., Calisher, C.H., Clark, G.G., Grimstad, P.R. and Bishop, D.H.L. (1981) Genotypic varieties of La Crosse virus isolated from different geographic regions of the continental United States and evidence for a naturally occurring intertypic recombinant La Crosse virus. Am. J. Epidemiol. 114, 112–131.

Obijeski, J.F., Bishop, D.H.L., Murphy, F.A. and Palmer, E.L.(1976a) Structural proteins of La Crosse virus. J. Virol. 19, 985–997.

Obijeski, J.F., Bishop, D.H.L., Palmer, E.L. and Murphy, F.A. (1976b) Segmented genome and nucleocapsid of La Crosse virus. J. Virol. 20, 664–675.

Shope, R.E., Rozhon, E.J. and Bishop, D.H.L. (1981) Role of the middle-sized bunyavirus RNA segment in mouse virulence. Virology 114, 273–276.

Thompson, W.H., Kalfayan, B. and Anslow, R.O.(1965) Isolation of California encephalitis group virus from a fatal human illness. Am. J. Epidemiol. 81, 245–253.